Available online at www.sciencedirect.com
ScienceDirect
Procedia Chemistry 1 (2009) 313–316
Procedia Chemistry
www.elsevier.com/locate/procedia

Proceedings of the Eurosensors XXIII conference

Novel chemico-mechanical approach towards long-term implantable glucose sensing

Christophe Boss[a,*], Eric Meurville[a], Jean-Michel Sallese[b], Peter Ryser[a]

[a]*Laboratory of Microengineering for Manufacturing, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland*
[b]*Institute of Electrical Engineering, Ecole Polytechnique Fédérale de Lausanne, 1015 Lausanne, Switzerland*

Abstract

The proof of concept of a continuously sensing affinity device based on the glucose-dependent viscosity of a sensitive solution containing dextran and Concanavalin A has been successfully demonstrated. The biosensor incorporates a piezoelectric diaphragm and a flow-resisting microchannel for viscosity detection, and a free-standing Anodic Alumina Oxide (AAO) porous nano-membrane as glucose selective interface. Extensive *in vitro* glucose measurements between two physiologically relevant glucose concentrations, 2 mM and 9 mM (respectively hypo- and hyperglycemia), were successfully performed during 4 days. To the best of our knowledge, such reversibility and stability of glucose measurement over time had not been reported yet.

Keywords: Continuous glucose monitoring; diabetes; affinity binding; biosensors; viscosity

1. Introduction

Diabetes mellitus is a metabolic disease resulting in abnormally high blood glucose levels. Serious long-term complications including cardiovascular disease, chronic renal failure and retinal damage are caused by chronic high glucose levels. Continuous glucose monitoring would allow a better diabetes management compared to current finger pricking, and would therefore reduce the risk of complications.

Existing minimally invasive systems are mostly based on electrochemical detection of enzyme-catalyzed reactions[1]. Electrochemical methods are sensitive but have several drawbacks when working in the subcutaneous tissue. The signal generated by the enzyme electrode depends on the glucose diffusion and therefore biofouling affects the device sensitivity. Furthermore, the presence of other electrochemically active solutes often produces inaccuracies. As a result, electrochemical sensors exhibit large drifts and require individual and frequent calibrations on the basis of parallel capillary blood analysis[1].

Alternative sensing methods, using competitive affinity binding of glucose, have shown great promise in overcoming these limitations[2-4]. Such methods are typically based on a solution of a polysaccharide cross-linked by a glucose-binding protein[2]. When glucose is added to the solution, it binds competitively to the glucose-binding

* Corresponding author. Tel.: +41(0)216937780; fax: +41(0)216933891.
E-mail address: Christophe.Boss@epfl.ch.

1876-6196/09/$– See front matter
doi:10.1016/j.proche.2009.07.078

protein, which can be detected via the resulting changes in solution properties, such as fluorescence or viscosity. Affinity sensing is more tolerant to biofouling, which results only in an increased stabilization time. Furthermore, affinity sensing does not degrade any glucose and is therefore not susceptible to electroactive interferents.

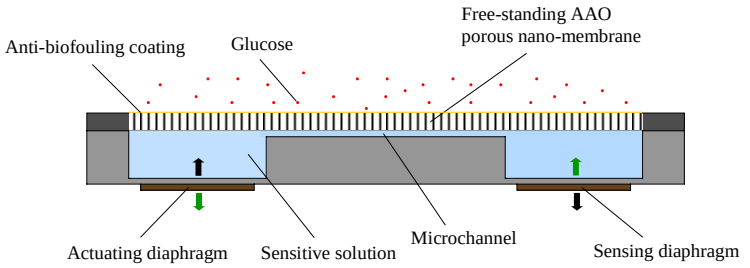

Fig. 1. Schematic view of the glucose biosensor.

2. Sensing principle

The affinity sensing solution is based on a protein, Concanavalin A (ConA), which specifically binds to glucose, and a branched polysaccharide, a high-molecular-weight dextran. At low glucose concentration, dextran molecules are crosslinked by binding to ConA, forming a viscous solution. When the glucose concentration increases, dextran is partially replaced by glucose at the binding sites of ConA. As a result, the network ConA-dextran is weakened, and the viscosity of the sensing solution decreases. This competitive affinity assay is reversible and highly sensitive. The change in viscosity can be one order of magnitude in the physiologically relevant glucose range (1-30 mM)[3].

The viscosity detection of the sensitive solution is based on a microchannel which exhibits a resistance to the flow circulating through. The viscometric glucose sensor, which is intended to be implanted subcutaneously, is illustrated in Fig. 1. The biosensor comprises two chambers (actuating and sensing chambers) containing the glucose sensitive solution, which communicate through the microchannel. The biosensor is hermetically sealed by a free standing AAO porous nano-membrane confining the dextran and ConA molecules, and letting the glucose contained in the interstitial fluid permeating through. The bottom of the two chambers consists of piezoelectric diaphragms which bend when applying a voltage or produce a voltage when deformed.

When applying a voltage, the actuating piezoelectric diaphragm bends, which generates a flow through the microchannel. As a result a deflection of the sensing piezoelectric diaphragm occurs, inducing a voltage which can be recorded. Due to its section, the microchannel exhibits a resistance to the flow which depends upon the viscosity of the sensitive solution. The delay between the excitation (burst or harmonic) and the deflection depends on the viscosity of the fluid. Such a biosensor may be fabricated using MEMS technology, bringing advantages such as miniaturization and reproducibility.

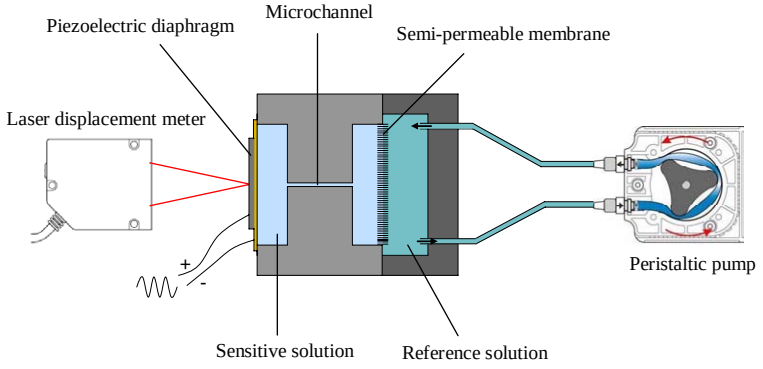

Fig. 2. Experimental *in vitro* setup.

3. Experimental setup

A macroscopic proof of concept of the biosensor has been realized. The demonstrator incorporates a 100 µm in diameter cylindrical microchannel and a 6.5 mm in diameter piezoelectric diaphragm for viscosity detection. The sensitive solution is confined in the biosensor using a 5-7 nm AAO porous nano-membrane as glucose selective interface (Synkera Technologies, Inc.). The biosensor has a volume of 80 µl and the surface of the selective interface is 12 mm^2. The displacement of the piezoelectric diaphragm is recorded using a specular-reflective laser displacement meter (LC-2420 from Keyence).

The experimental *in vitro* setup is represented in Fig. 2. The interstitial fluid is mimicked by a reference which is an isotonic solution of the sensitive fluid, but without dextran and ConA. A peristaltic pump makes the reference solution circulating in order to maintain a constant glucose concentration outside the biosensor. The glucose concentration is increased by injecting small amounts of concentrated (2M) D-glucose solution with a microliter syringe. The reference solution is flushed and replaced when a decrease in glucose concentration is desired. The whole setup is located in a thermally regulated chamber, as the viscosity of the sensitive fluid is strongly temperature dependant.

4. Results and discussion

The deflection of the piezoelectric diaphragm was characterized when applying a slowly varying sinusoidal voltage. With a voltage of 25 V, the center of the piezoelectric diaphragm was deflected of 4.3 µm. Such a deflection displaces a volume of 0.04 µl, which corresponds to a fluid displacement of 5.2 mm in the microchannel, enabling sensitive viscosity detection as well as efficient mixing between the two chambers. The demonstrator was then characterized using 5 viscosity reference standards (Paragon Scientific Ltd), exhibiting dynamic viscosities ranging from 3.65 mPa s to 17.25 mPa s at 20°C. 20 measurements were recorded for each viscosity, showing a good accuracy (mean standard deviation of 0.18%) and stability.

A long-term measurement was performed during 4 days at 25°C, between two physiologically relevant glucose concentrations, 2 mM and 9 mM (Fig. 3). A sinusoidal voltage with a frequency of 0.5 Hz and an amplitude of 25 V was applied to the piezoelectric diaphragm. The measured phase shift was stable and reversible throughout the experiment. The reversibility of the sensitive solution had already been shown by Ballerstädt et al. for one cycle of glucose change during a short period[2]. But the sensitive solution confined by a semi-permeable membrane was not stable, thus hindering from getting long-term reversible results. Due to its small hydrodynamic radius (3.3 nm in dimer configuration), ConA leakage was though to be responsible of the sensitive solution instability. The 5-7 nm AAO porous nano-membrane completely confines dextran and ConA molecules inside the demonstrator, resulting in a long-term stability of measurements.

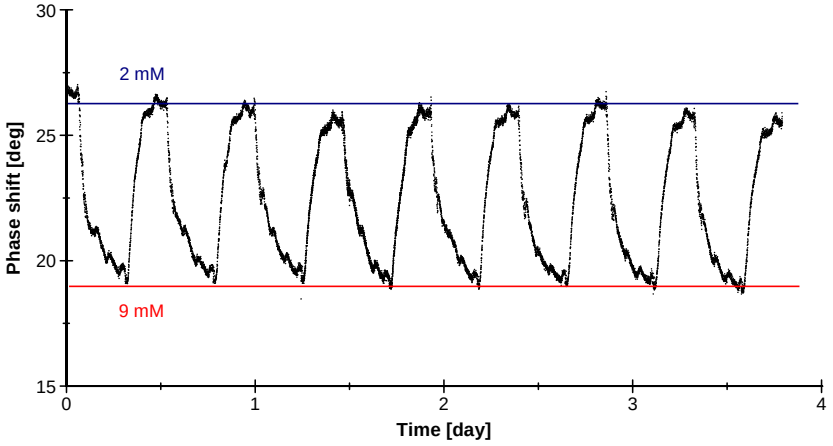

Fig. 3. *In vitro* glucose concentration measurement at 25°C, between two physiologically relevant glucose concentrations, 2 mM and 9 mM, showing excellent stability and reversibility over 4 days.

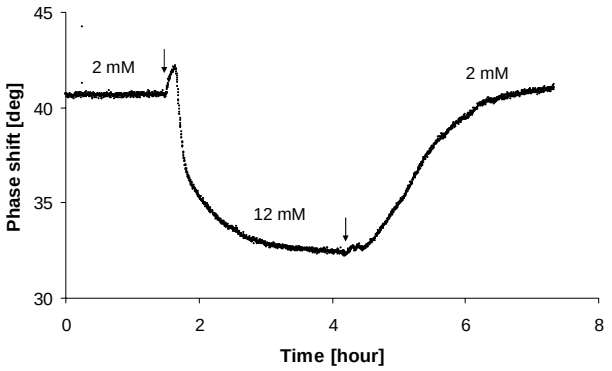

Fig. 4. *In vitro* glucose concentration measurement at 37°C, between two physiologically relevant glucose concentrations, 2 mM and 12 mM. Arrows indicate changes of glucose concentration in the reference solution.

Glucose measurements were then performed at 37°C, to evaluate the response time of the demonstrator at physiological temperature. A glucose concentration measurement between 2 mM and 12 mM is shown in Fig. 4. A sinusoidal voltage with a frequency of 2 Hz and an amplitude of 25 V was applied to the piezoelectric diaphragm. Curve fittings with a decreasing exponential were performed, which gave a time constant of 20.4 min when increasing the glucose concentration, and a time constant of 73.2 min when decreasing the glucose concentration. A longer time constant when decreasing the glucose concentration has also been observed for other similar experiments, and might be explained by the smaller mobility of glucose molecules in the viscous sensitive solution than in the reference solution. The response time of the demonstrator is very long, due to the large volume of sensitive solution and particularly to the small semi-permeable surface compared to the volume of the sensitive solution. Currently, a new demonstrator is being developed, which will address the long equilibration time issue through size reduction and semi-permeable surface increase. Time constants between 3-10 min are expected.

5. Conclusion

A proof of concept for a long-term implantable glucose sensor was demonstrated. Reversible and stable measurements, between two physiologically relevant glucose concentration, 2 mM and 9 mM, were successfully performed during 4 days. The viscometric sensitive solution confined by a nanoporous AAO membrane was shown to be stable and therefore suitable for long-term continuous glucose monitoring. Moreover, the viscosity measurement principle, based on two piezoelectric diaphragms, would be suitable for long-term implantation due to its low-power consumption. This demonstrator represents a first step towards long-term implantable glucose sensing using a non-consumptive viscometric affinity assay. Miniaturization of the device using MEMS technology, and biofouling and fibrous encapsulation following the subcutaneous implantation are the remaining critical hindrances, and will be the object of future studies.

Acknowledgements

The authors would like to thank Dr. N. Takano who has kindly prepared the glucose sensitive solution.

References

1. Klonoff DC. Continuous Glucose Monitoring: Roadmap for 21st century diabetes therapy. Diabetes Care 2005;28:1231–39.
2. Ballerstädt R, Ehwald R. Suitability of Aqueous Dispersions of Dextran and Concanavalin-a for Glucose Sensing in Different Variants of the Affinity Sensor. Biosensors & Bioelectronics 1994;9:557–67.
3. Ehwald R, Ballerstädt R, Dautzenberg H. Viscosimetric Affinity Assay. Anal Biochem 1996;234:1–8.
4. Zhao Y, Li S, Davidson A, Yang B, Wang Q, Lin Q. A MEMS viscometric sensor for continuous glucose monitoring. J Micromech Microeng 2007;17:2528–37.